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(54) Title: COMPOSITIONS COMPRISING VANADIUM COMPOUNDS FOR USE IN THE TREATMENT OF PROLIFERATIVE DISORDERS AND ARTHROPATHIES

(57) **Abstract**

The present invention relates to the use of vanadium compounds as antiproliferative and anti-metastatic agents, to treat arthropathy and drug resistant tumors in animals; to compositions containing vanadium compounds adapted for such uses; to methods for the treatment of proliferative disorders, to methods of reducing the ability of a tumor to metastasize, to methods for treating drug resistant tumors and to methods for treating arthropathies, such as arthritis.

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COMPOSITIONS COMPRISING VANADIUM COMPOUNDS FOR USE IN THE TREATMENT OF PROLIFERATIVE DISORDERS AND ARTHROPATHIES

FIELD OF THE INVENTION

The present invention relates to the use of vanadium compounds as antiproliferative and anti-metastatic agents, to treat arthropathy and drug resistant tumors in animals; to compositions containing vanadium compounds adapted for such uses; to methods for the treatment of proliferative disorders, to methods of reducing the ability of a tumor to metastasize, to methods for treating drug resistant tumors and to methods for treating arthropathies, such as arthritis.

BACKGROUND OF THE INVENTION

Cancer is a global problem which affects an estimated 5.9 million people worldwide annually. There are many types of cancer, some of the most common in North America include breast, lung, colon and lymphatic cancer. Although chemotherapy has had a positive impact on the survival rate of cancer patients in the last 30 years, most human cancers are, or become resistant to chemotherapy. Thus, there is a tremendous need for anticancer drugs which are more effective and which can act on drug resistant tumors.

Two important features of cancer cells is their ability to proliferate abnormally leading to tumor formation and growth, and to invade other tissues leading to metastases. It is thought that genetic damage to specific genes is responsible for the transformation of cells and the development of cancer in humans. The genetic damage found in human cancer cells can be divided into two types. One of these involves the mutation of oncogenes which results in continuous proto-oncogene activation. The second involves the mutation of tumor suppressor genes which results in the loss of their function. Genetic damage to proto-oncogenes or to tumor suppressor genes leads to oncogene activation in the absence of stimuli and to uncontrolled cellular proliferation. Damage has been found to one or another proto-oncogenes and tumor suppressor genes with some consistency in a variety of human malignancies.

Two oncogenic transcription factors, fos and jun, have been shown to be involved and required for the induction of genes involved in cellular proliferation and in particular, in cellular proliferation in many tumor cell lines. Inhibition of the expression of these two genes leads to the inhibition of cellular proliferation.

One of the most life threatening aspects of cancer is the development of metastases. Generally, most solid tumors can be removed surgically from the primary site resulting in a local cure. However, if the cancer cells have invaded vascular channels and metastasized to a different organ, then the likelihood of a complete cure is reduced. Thus, agents which reduce the metastatic properties of cancer cells would be beneficial for the treatment of cancer.

The cellular processes thought to play an important role in metastases include; increased cellular attachment, tumor cell proteolysis of host tissue, tumor cell locomotion and

colony formation. These processes occur in a sequential order. First, tumor cells attach to the basement membrane through their surface receptors of integrin and non-integrin types to ligands such as collagen, laminin and fibronectin in the basement membrane. After attachment, a localized zone of lysis of the basement membrane occurs at the point of cell attachment. The tumor cells produce and secrete degradative enzymes, such as collagenase and gelatinase, which degrade the basement membrane and allow the infiltration and locomotion of tumor cells into the host organ. There is a positive association between tumor aggressiveness and the ability of cells to produce a group of enzymes, matrix metalloproteases, involved in the invasive process. Inhibition of certain proteases, such as metalloproteases or serine proteases, have been shown to prevent invasion and metastasis (Alvarez et al. 1990. J. Natl. Cancer Inst. 82: 589-595; Schultz et al 1988, Cancer Res. 48, 5539-5545; and, Wang & Stearns 1988, Cancer Res. 48, 6262-6271). Metalloproteases, such as collagenase have also been associated with cartilage erosion and pathology in arthropathies, such as arthritis.

Inflammatory arthritis is a serious health problem in developed countries, particularly given the increasing number of aged individuals. For example, one form of inflammatory arthritis, rheumatoid arthritis (RA) is a multisystem chronic, relapsing, inflammatory disease of unknown cause. Although many organs can be affected, RA is basically a severe form of chronic synovitis that sometimes leads to destruction and ankylosis of affected joints (taken from *Robbins Pathological Basis of Disease*, by R.S. Cotran, V. Kumar, and S.L. Robbins, W.B. Saunders Co., 1989). Pathologically the disease is characterized by a marked thickening of the synovial membrane which forms villous projections that extend into the joint space, multilayering of the synoviocyte lining (synoviocyte proliferation), infiltration of the synovial membrane with white blood cells (macrophages, lymphocytes, plasma cells, and lymphoid follicles; called an "inflammatory synovitis"), and deposition of fibrin with cellular necrosis within the synovium. The tissue formed as a result of this process is called pannus and eventually the pannus grows to fill the joint space. The pannus develops an extensive network of new blood vessels through the process of angiogenesis which is essential to the evolution of the synovitis. Release of digestive enzymes [matrix metalloproteinases (e.g., collagenase, stromelysin)] and other mediators of the inflammatory process (e.g. hydrogen, peroxide, superoxides, lysosomal enzymes, and products of arachadonic acid metabolism) from the cells of the pannus tissue leads to the progressive destruction of the cartilage tissue. The pannus invades the articular cartilage leading to erosions and fragmentation of the cartilage tissue. Eventually there is erosion of the subchondral bone with fibrous ankylosis and ultimately bony ankylosis, of the involved joint.

It is generally believed, but not conclusively proven, that RA is an autoimmune disease, and that many different arthriogenic stimuli activate the immune response in the

immunogenetically susceptible host. Both exogenous infectious agents (Ebstein-Barr Virus, Rubella virus, Cytomegalovirus, Herpes Virus, Human T-cell Lymphotropic Virus, Mycoplasma, and others) and endogenous proteins (collagen, proteoglycans, altered immunoglobulin) have been implicated as the causative agent which triggers an inappropriate host immune response. Regardless of the inciting agent, autoimmunity plays a role in the progression of the disease. In particular, the relevant antigen is ingested by antigen-presenting cells (macrophages or dendritic cells in the synovial membrane), processed, and presented to T lymphocytes. The T cells initiate a cellular immune response and stimulate the proliferation and differentiation of B lymphocytes into plasma cells. The end result is the production of an excessive inappropriate immune response directed against the host tissues [e.g. antibodies directed against Type II collagen, antibodies directed against the Fc portion of autologous IgG (called "Rheumatoid Factor")]. This further amplifies the immune response and hastens the destruction of the cartilage tissue. Once this cascade is initiated numerous mediators of cartilage destruction are responsible for the progression of rheumatoid arthritis.

Rheumatoid arthritis is associated with an inflammatory response and cell proliferation. Neutrophils are found in abundance in the synovial fluid, but only in small numbers in the synovial membrane itself. It is estimated that more than 1 billion neutrophils enter a moderately inflamed rheumatoid knee joint each day (Hollingsworth et al., 1967) and remain there because no pathway exists by which they can leave the joint. These cells release reactive free radicals and lysosomal enzymes which degrade the cartilage tissue. Other PMN products such as prostaglandins and leukotrienes augment an inflammatory response and recruit more inflammatory cells into the joint tissue.

Lymphocytes, particularly T cells, are present in abundance in the diseased synovial tissue. Activated T cells produce a variety of lymphokines and cooperate with B cells to produce autoantibodies. T cell products result in the activation of macrophages, a cell which is thought to have an important role in the pathology of the disease. The macrophages produce a variety of destructive lysosomal enzymes, prostaglandins, and monokines and are also capable of stimulating angiogenesis. One of the more important monokines secreted by macrophages is IL-1. Briefly, IL-1 is known to: stimulate synthesis and release of collagenase by synoviocytes and synovial fibroblasts, inhibit proteoglycan synthesis by chondrocytes, activate osteoclasts, induce changes in the endothelium of the synovial vasculature, and act as a chemoattractant for lymphocytes and neutrophils.

During the development of RA, the synovial lining cells become activated by products of inflammation or through phagocytosis of immune complexes. Several subtypes of synovial lining cells have been identified and all of them become intensely activated and undergo excessive hyperplasia and growth when stimulated. As the synovial tissue organizes to form a pannus, the number of synoviocytes, blood vessels, connective tissue elements, and

inflammatory cells increases to form a mass 100 times its original size. In many ways, the synovitis in rheumatoid arthritis behaves much like a localized neoplasia (Harris, 1990). In fact, cultured rheumatoid synovial cells develop the phenotype characteristics of anchorage-independent growth usually associated with neoplastic cells if they are given sufficient platelet derived growth factor (Lafyatis et al, 1989). In addition, the synoviocytes also produce large amounts of collagenase, stromelysin, prostaglandins, and Interleukin-1.

The tumor-like proliferation of the cells of the synovial connective tissue stroma (synoviocytes, fibroblast-like cells and neovascular tissue) produces a pannus with many features of a localized malignancy. Supporting this tumor analogy are several findings: the pannus expresses high levels of oncoproteins such as c-myc and c-fos, produces metalloproteinases to facilitate surrounding tissue invasion, and expresses cytosketetal markers characteristic of poorly differentiated mesenchymal tissue (e.g. vimentin); synoviocytes *in vitro* grow rapidly, do not contact inhibit, form foci, and can be grown under anchorage-independent conditions in soft agarose; and pannus tissue is capable of inducing the growth of a supporting vasculature (i.e. angiogenesis). All these findings are suggestive of a tissue in which normal growth regulation has been lost.

Irreparable degradation of the cartilage extracellular matrix is believed to be largely due to the enzymatic action of matrix metalloproteinases on the components of the cartilage matrix. Although numerous other enzymes are likely involved in the development of RA, collagenase (MMP-1) and stromelysin (MMP-3) play an important role (Vincetti et al., 1994) in disease progression. These enzymes are capable of degrading type 11 collagen and proteoglycans, respectively; the 2 major extracellular components of cartilage tissue. Cytokines such as IL-1, epidermal growth factor (EGF), platelet-derive growth factor, and tumor necrosis factor are all potent stimulators of collagenase and stromelysin production. As described above, numerous cell types found in the arthritic joint (white blood cells, synoviocytes, endothelial cells, and chondrocytes) are capable of synthesizing and secreting MMPS.

In proliferating rheumatoid synovial tissue, collagenase and stromelysin become the major gene products of the pannus and may comprise as much as 2% of the messenger RNAs produced by the synovial fibroblasts. Increased levels of collagenase and stromelysin are present in the cartilage of patients with RA and the level of enzyme activity in the joint correlates well with the severity of the lesion (Martel-Pelletier et al., 1993; Walakovitis et al., 1992.

The development of an extensive network of new blood vessels is essential to the development of the synovitis present in rheumatoid arthritis (Harris 1990, Folkman et al., 1989; Sano et al., 1990). Several local mediators such as platelet derived growth factor (PDGF), TGF-ß, and fibroblast growth factor (FGF) are likely responsible for the induction and perpetuation of neovasularization within the synovium. Pannus tissue composed of new

capillaries and synovial connective tissue invades and destroys the articular cartilage. The migrating angiogenic vessels themselves produce and secrete increased levels of metalloproteinases such as collagenase and stromelysin capable of degrading the cartilage matrix (Case et al., 1989). The newly formed vessels are also quite "leaky" with gaps present between the microvascular endothelial cells. This facilitates the exudation of plasma proteins into the synovium (which increases swelling), enhances WBCs movement from the circulation into the pannus tissue (which increases inflammation), and leads to the perivascular accumulation of mononuclear inflammatory cells (Wilder et al., 1991).

Orthovanadate has been found to increase phosphotyrosine levels and inhibit collagenase production by chondrocytes, suggesting that the two processes are linked (Cruz et al., Biochem J. (1990) 269, 717-721).

Ionic vanadium compounds such as vanadyl or vanadate salts in combination with thiosulphate or sulfite compounds have been reported to be useful for treating malignant tumors, arteriosclerosis and mental syndromes in the elderly ((U.S. Patent Serial No. 5,045,316 to Kaplan). Kaplan discloses a daily dose ranging from 0.0043 mg/kg to 0.14 mg/kg of vanadyl or vanadate salts. No mechanism for the action of vanadate and thiosulphate in the disclosed treatments is provided by Kaplan. In the background of the Kaplan patent it is disclosed that others have reported that vanadium salts have an antineoplastic effect and dietary vanadyl sulphate has been reported to inhibit chemically induced mammary carcinogenesis in rats.

Saxena et al. (Biochem. Pharmacology 45(3): 539-542, 1993) examined the *in vivo* effects of vanadate on the antioxidant status of control and alloxan diabetic rat livers. Diabetic rats were administered 0.6 mg sodium orthovanadate/ml in drinking water. It should be noted that the present inventor has found that oral administration of orthovanadate to animals at 0.5 mg/ml results in gastric toxicity (See Example 9 herein).

Antioxidants such as ß-carotene, a-tocopherol, vitamin E, vitamin C, and glutathione have been reported to have anticancer activity (G. Shklar et al. Nutrition and Cancer, 1993, p.145). It has also been disclosed that a mixture of antioxidants (ß-carotene, dl-a-tocopherol acid succinate (vitamin E), vitamin C, and reduced glutathione) was very effective in preventing carcinogenesis in an *in vivo* cancer model and was more effective than the individual components of the mixture as cancer chemopreventive agents.

SUMMARY OF THE INVENTION

The present inventor has found that certain classes of vanadium complexes are particularly useful in reducing cell proliferation. The classes of vanadium complexes include (a) vanadate complexes such as metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate; and (c) vanadyl

complexes including coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate and vanadyl sulfates. The present inventor has shown that a specific organo-vanadium complex, namely bis(ethylmaltolato) oxovandium (BEOV), is most efficacious and it has the advantage of being less toxic and it is a potent inhibitor of protein tyrosine phosphatase.

Accordingly the invention contemplates a method for reducing hydrogen peroxide and/or superoxides thereby reducing cell proliferation and metalloprotease expression comprising administering a vanadium complex selected from the group consisting of (a) vanadate complexes such as metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate; and (c) vanadyl complexes including coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate and vanadyl sulfates. Preferably the vanadium complex is an organo-vanadium complex, most preferably, bis(ethylmaltolato) oxovandium (BEOV).

The invention also contemplates a pharmaceutical composition for the treatment of proliferative disorders comprising an amount of a vanadium complex effective to reduce cell proliferation, and one or more of a pharmaceutically acceptable carrier, diluent, or excipient, wherein the vanadium complex is selected from the group consisting of (a) vanadate complexes such as metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six-membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate; and (c) vanadyl complexes including coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate and vanadyl sulfates. Preferably the vanadium complex is an organo-vanadium complex, most preferably, bis(ethylmaltolato) oxovandium (BEOV). In a preferred embodiment of the invention, the pharmaceutical composition is used to reduce tumor growth. The invention further contemplates a method for the treatment of a proliferative disorder comprising administering an amount of a selected vanadium complex effective to reduce cell proliferation.

The invention also relates to a method for reducing or inhibiting the growth of drug resistant tumors comprising administering an amount of a selected vanadium complex effective to reduce or inhibit the growth of drug resistant tumors. The invention further contemplates a method for reducing or inhibiting metastases comprising administering an amount of a selected vanadium complex effective to reduce or inhibit metastases.

The invention also contemplates a composition comprising a vanadium complex, and optionally at least one antioxidant, preferably N-acetylcysteine, which enhances the antiproliferative and anti-metastatic effects of the vanadium complex and reduces cell

proliferation and metastases. Methods of treating and preventing proliferative disorders, treating drug resistant tumors, and reducing metastases using this composition are also provided.

The present inventor has also shown that vanadium compounds such as vanadate or vanadyl compounds and complexes, derivatives and analogues thereof, for example, orthovanadate bis(ethylmaltolato) oxovanadium (BEOV), ammonium bis vanadate (ABOV) and bis(methylmaltolato) oxovanadium (BMOV) inhibit the proliferation of cells, such as synoviocytes, and also inhibit the production of metalloproteases, such as collagenase. In particular, the present inventor has shown that orthovanadate and N-acetylcysteine inhibit the production of collagenase in chondrocytes *in vitro* and has further shown that a vanadate compound BMOV and N-acetylcysteine regress arthritis in rats having collagen induced arthritis. The present inventor has also concluded that systemic administration and in particular systemic administration of the vanadium compound orthovanadate bis(ethylmaltolato) oxovanadium (BEOV) are particularly useful in the treatment of athropathies, such as arthritis.

Accordingly, the invention also contemplates a method for treating a mammal having an arthropathy, comprising systemically administering to the mammal an amount of a vanadium compound, in particular a vanadate or a vanadyl compound effective to reduce or inhibit the arthropathy and, optionally a pharmaceutically acceptable vehicle. In a particular embodiment, the arthropathy is an arthritis, such as rheumatoid arthritis. In a further particular embodiment, the vanadate compound is BEOV. The method may further comprise administering an antioxidant to the mammal, preferably N-acetylcysteine.

In an embodiment of the invention a method is provided for treating a mammal having an arthropathy, comprising systemically administering to the mammal an amount of a vanadium compound effective to reduce or inhibit the arthropathy, with the proviso that the vanadium compound is not bis(methylmaltolato) oxovanadium (BMOV).

Also provided is a method of reducing metalloprotease expression. In an embodiment, a method is provided for reducing collagenase expression in an arthritic joint of a mammal comprising administering an amount of a vanadium compound such as a vanadate or vanadyl compound, and a pharmaceutically acceptable vehicle in an amount effective to reduce the collagenase expression. In a preferred embodiment, the pharmaceutical composition further comprises at least one antioxidant. In a particular embodiment, the vanadate compound is BEOV and the antioxidant is N-acetylcysteine.

A method is also contemplated for reducing proliferation of synoviocytes in a mammal comprising administering a vanadium compound such as a vanadate or vanadyl compound.

In a still further embodiment, the invention provides a pharmaceutical composition for use as an anti-arthritic agent comprising a vanadium compound such as a

vanadate or vanadyl compound; at least one antioxidant, and a pharmaceutically acceptable vehicle. In a particular embodiment, the vanadate compound is BEOV and the antioxidant is N-acetylcysteine.

The use of a vanadium compound such as a vanadate or vanadyl compound and optionally at least one antioxidant in the preparation of a pharmaceutical for treating arthritis is also contemplated.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, reference is made herein to various publications, which are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Further details of the invention are described below with the help of the examples illustrated in the accompanying drawings in which:

Figure 1 shows the chemical structure of bis(methylmaltolato) oxovanadium, (BMOV);

Figure 2 is a graph showing the daily mean arthritis score;

Figure 3 is an autoradiograph showing the X-rays of control (left panel) and vanadate treated experimental (right panel) rat limbs;

Figure 4A is a scanning electron micrograph of the articular cartilage of an arthritic control rat;

Figure 4B is a scanning electron micrograph of the articular cartilage of a BMOV-treated arthritic rat;

Figure 4C is a scanning electron micrograph of the articular cartilage of an arthritic control rat;

Figure 4D is a scanning electron micrograph of the articular cartilage of a BMOV-treated arthritic rat;

Figure 4E is a scanning electron micrograph of the articular cartilage of an arthritic control rat;

Figure 4F is a scanning electron micrograph of the articular cartilage of a BMOV-treated arthritic rat;

Figure 5A is a transmission electron micrograph showing trochlear articular cartilage from naive rats;

Figure 5B is a transmission electron micrograph showing trochlear articular cartilage from arthritic control rats;

Figure 5C is a transmission electron micrograph showing trochlear articular cartilage from BMOV-treated rats;

Figure 6 is a Northern blot of collagenase, stromelysin, and IL-1 expression;

Figure 7 is a graph showing the effect of BEOV on tumor growth;

Figure 8 is a graph showing the effect of orthovanadate concentration on tumor weight; and

Figure 9 is a graph showing the effect of BMOV concentration on tumor weight.

DETAILED DESCRIPTION OF THE INVENTION

I. Proliferative Disorder, Metastasis, and Drug Resistant Tumor Applications

The present invention relates to a method of modulating fos and jun expression by regulating concentrations of hydrogen peroxide using selected vanadium complexes. Increasing the concentrations of hydrogen peroxide should result in increased expression of fos and jun and accordingly an increase in cell proliferation. An increase in cell proliferation would be useful in the treatment of conditions involving damaged cells and in particular may be useful in treating conditions in which degeneration of tissue occurs such as bone resorption, inflammatory disease, degenerative disorders of the central nervous system, and for promoting wound healing. Decreasing the concentrations of hydrogen peroxide should result in decreased expression of fos and jun and accordingly a decrease in cell proliferation and expression of metalloproteases. A decrease in cell proliferation and metalloproteases would be useful in treating proliferative conditions such as cancer and arthropathy.

In accordance with an embodiment of the invention compounds are used to reduce hydrogen peroxide and/or superoxides to thus effect a reduction in cell proliferation and in metalloprotease expression. The compounds are vanadium complexes including metavanadate and orthovanadate complexes, such as ammonium metavanadate, sodium metavanadate and sodium orthovanadate. Suitable vanadium complexes also include organo-vanadium compounds where, for example, vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate. Preferably, bis(methylmaltolato)oxovanadium (BMOV), Bis(ethylmaltolato) oxovandium (BEOV), ammonium bisvanadate (ABOV) organo-vanadium compounds are used in the present invention.

Suitable vanadium complexes also include vanadyl complexes, for example, coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate and vanadyl sulfates, including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

A selected vanadium complex may be tested for its ability to reduce hydrogen peroxide, its ability to effect growth of proliferating cell lines, non-proliferating cell lines, and drug resistant cell lines, and its ability to inhibit tumor growth or metastases in animal models following the methods described herein.

The composition of the invention may contain one of more antioxidants in combination with a vanadium complex. The antioxidant(s) are selected based on their ability to increase the efficacy of the vanadium complexes and reduce toxicity on normal cells using

the methods described herein. Suitable antioxidants for use in the enhancing composition of the invention include N-acetylcysteine, glutathione, Vitamin E (alpha-tocopherol), Vitamin C (ascorbic acid), beta-carotene, ergothioneine, zinc, selenium, copper, manganese, flavonoids and estrogens, or derivatives thereof, preferably N-acetylcysteine.

The administration of vanadium complexes, and optionally one or more antioxidants, in the forms and modes described herein reduces hydrogen peroxide to effect a reduction in cell proliferation, and also reduces metastases of tumors. Thus, the compositions may be used for the treatment of proliferative disorders including various forms of cancer such as leukemias, lymphomas (Hodgkins and non-Hodgkins), sarcomas, melanomas, adenomas, carcinomas of solid tissue, hypoxic tumors, squamous cell carcinomas of the mouth, throat, larynx, and lung, breast, ovarian, colon, genitourinary cancers such as cervical and bladder cancer, hematopoietic cancers, human glioma and astrocytoma primary tumors, head and neck cancers, and nervous system cancers, benign lesions such as papillomas, arthrosclerosis, angiogenesis, and viral infections, in particular HIV infections.

Vanadate complexes, and optionally one or more antioxidants, in the compositions described herein may also be used to treat drug resistant tumors. Examples of drug resistant tumors are tumors expressing high levels of P-glycoprotein which is known to confer resistance to multiple anticancer drugs such as colchicine, vinblastine and doxorubicin, or tumors expressing the multi-drug resistance protein as described in R. Deeley et al., Science, 258:1650-1654, 1992.

The compositions of the invention contain vanadium complexes, and optionally one or more antioxidants, either alone or together with other substances. Such pharmaceutical compositions can be for topical, parenteral (intravenous, subcutaneous, intramuscular or intramedullary) or local use. Preferably, a mode of administration is used which results in a slow continuous release of the active substances. This may be achieved by intravenous administration, subcutaneous administration, or using control release mechanisms such as implants or pumps. Control release methods generally use control release polymers and the release of the active ingredient is based on solubility properties, and the pore size of the polymers and active ingredients. The vanadium complexes may also be administered in pastes, such as thermopastes, in microspheres or palla beads.

In the case of parenteral administration, solutions, suspensions, emulsions or powders of the vanadium complexes, and optionally antioxidant(s) can be employed, using one or more pharmaceutically acceptable excipients or diluents, suitable for the aforesaid uses and with an osmolarity which is compatible with the physiological fluids. For local use, those preparations in the form of creams or ointments for topical use or in the form of sprays should be considered.

The preparations of the invention can be intended for administration to humans and various other mammals, such as ovines, bovines, equines, swine, canines, and felines.

The amount of a vanadium complex effective to reduce cell proliferation, and/or to reduce metastases or treat drug resistant tumors is the minimum dose adequate to achieve a reduction in cell proliferation, reduction or inhibition of metastases, and/or growth of drug resistant tumors. For example, a dose which results in a concentration of the complex in extracellular body fluids such as serum, synovial fluid or cerebral spinal fluid, of at least about 5μM, preferably 5-50μM, most preferably 10-30μM, may be used to reduce cell proliferation and accordingly provide for effective treatment of proliferative disorders. Generally, a dose of at least 0.2 mg/kg, preferably 0.2 mg/kg to 25 mg/Kg, most preferably 0.2 mg/kg to 20 mg/Kg will provide an appropriate concentration in humans and other mammals. In an embodiment of the invention a dose of at least 1.0 mg/kg and preferably between 1.0 mg/kg and 25 mg/kg will provide an optimum dosage in humans and other mammals. The above-mentioned doses may be used to reduce metastases and treat drug resistant tumors. The selected doses will also depend on individual needs and the mode of administration.

It will be appreciated that standard procedures may be used to quantitate the concentration of the vanadium complexes in extracellular body fluids.

When the vanadium complex is used in combination with one or more antioxidants, the doses of the vanadium complex and the antioxidant(s) are selected so that the vanadium complex and antioxidant(s) alone would not show a full effect. Generally, the effective doses of the vanadium complex and the antioxidant(s) are the minimum doses adequate for enhanced antiproliferative or anti-metastatic effects. The vanadium complex and antioxidant(s) may be administered concurrently, separately, or sequentially.

The vanadium complex and antioxidant may be prepared and administered as a complex. For example, a vanadium complex may be conjugated with glutathione or N-acetylcysteine.

In an embodiment of the invention, a dose of a vanadium complex is administered which provides a concentration of the compound in extracellular body fluids such as serum, synovial fluid or cerebral spinal fluid, of at least 5μM, preferably 5-50μM, most preferably 10-30μM. N-acetylcysteine is administered prior to, (preferably 20 minutes prior to), and during administration of the vanadium complex, at a dose which provides a concentration of the compound of between 0.5mM to 15.0mM, preferably 5mM to 12.5 mM. Generally, a dose of between 40.0 mg/kg to 1000 mg/Kg of N-acetylcysteine will provide an appropriate concentration in humans and other mammals.

The compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the pharmaceutical compositions include,

albeit not exclusively, solutions of the vanadium complexes in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The compositions and treatments are indicated as therapeutic agents or treatments either alone or in conjunction with other therapeutic agents or other forms of treatment. In particular, the compositions and treatments described herein may be used to reduce toxicity of other therapeutic agents. For example the compositions of the invention may be used in combination with radiotherapy or chemotherapy, such as multi-drug chemotherapy for Hodgkins disease or combination radiotherapy, and chemotherapy for treatment of breast cancer.

II. Arthropathy Applications

The invention contemplates a method or composition for treating a mammal having an arthropathy, comprising administering to the mammal an amount of a vanadium compound or an analogue, derivative or complex thereof, effective to reduce or inhibit the arthropathy and optionally, a pharmaceutically acceptable vehicle. Arthropathy includes inflammatory and degenerative diseases of joints such as arthritis, rheumatoid arthritis, osteoarthritis, enteropathic arthritis, gouty arthritis, Jaccoud's arthritis and neuropathic arthritis. The arthropathy is considered to be reduced if at least one symptom of the arthropathy is beneficially altered. Symptoms of arthropathy are known in the art and include, for example, redness, swelling, pain, stiffness, reduced mobility, joint changes on radiographic examination etc. Increased levels of metalloproteases are associated with pathology in osteoarthritis.

At least one antioxidant, such as N-acetylcysteine, may also be administered to the mammal simultaneously with, prior to, or subsequent to the administration of the vanadium compound. Therefore, in an embodiment of the invention a method for treating arthritis in a mammal is provided comprising administering a pharmaceutical composition consisting essentially of an amount of a vanadium compound; at least one antioxidant and; a pharmaceutically acceptable vehicle. The treatment may be used to decrease cell proliferation, the production of metalloproteases, cartilage destruction and erosion of the synovium in the joints of the arthritic mammals. The destruction of cartilage matrix of the joints is a major feature of the pathology of arthritis.

The compounds used in the arthropathy methods and compositions of the invention are vanadium compounds, or derivatives or analogues thereof. Suitable compounds for use in the present invention are oxidative forms of vanadate, preferably orthovanadate. Derivatives of vanadate compounds, preferably pharmaceutically acceptable salts, esters and complexes of vanadate compounds including potassium and sodium salts, and amino acid, carbohydrate and fatty acid complexes, for example, vanadate complexed with cysteine, dihydroxamate, and glucuronate may also be used in the present invention. Representatives of

vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl compounds. Suitable vanadium complexes include metavanadate and orthovanadate complexes, such as ammonium metavanadate, sodium metavanadate and sodium orthovanadate.

Suitable vanadium complexes also include organo-vanadium compounds where, for example, vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate. Examples of these complexes are bis(methylmaltolato)oxovanadium (BMOV), Bis(ethylmaltolato) oxovandium (BEOV), and ammonium bisvanadate (ABOV) organo-vanadium compounds.

Suitable vanadium complexes also include vandyl complexes, for example, coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate and vanadyl sulfates, including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Suitable analogues may be selected based upon their functional similarity to vanadium compounds. Examples of such compounds include metal ions such as iron, titanium, molybdenum, tungsten, tantalum, cobalt, stannum, glutathione, and diphenyl iodonium, and complexes thereof, and nickel and chromium complexes. In particular, the following complexes may be used in the present invention: ammonium tungstate, sodium tungstate dihydrate, tungstate acid, tungsten (IV) oxide, tungsten (VI) oxide, molybate and its hydrates, molybdenum oxide, ammonium molybdate and its hydrates, sodium molybdate and its hydrates, potassium molybdate and its hydrates, molybdenum (IV) oxide molybdenum (VI) oxide, molybdic acid, molybdenum acetylacetonate, and suitable yungsten and molybdenum complexes including hydroxo derivatives derived from glycerol, tartaric acid, and sugars. Analogues of vanadium compounds may also be selected based upon their three dimensional structural similarity to vanadium compounds. For example, the vanadyl forms of vanadium may be used in the present invention, preferably vanadyl sulphate.

In a preferred embodiment, bis(ethylmaltolato) oxovandium (BEOV) and, ammonium bisvanadate (ABOV) organo-vanadium compounds are used in the present invention. Most preferably, bis(ethylmaltolato) oxovandium (BEOV) which has low toxicity and is a potent inhibitor of protein tyrosine phosphatase is used in the present invention.

A selected derivative or analogue of a vanadium compound may be tested for its ability to effect growth of synoviocytes, and its ability to regress arthritis in animal models following the methods described herein.

The arthropathy compositions of the invention may contain one of more antioxidants in combination with a vanadium compound or analogue or derivative thereof. The antioxidant(s) are selected based on their ability to increase the efficacy of the vanadium compounds and reduce toxicity on normal cells using the methods described herein.

Suitable antioxidants for use in the invention include N-acetylcysteine, glutathione, Vitamin E (alpha-tocopherol), Vitamin C (ascorbic acid), beta-carotene, ergothioneine, zinc, selenium, copper, manganese, flavonoids and estrogens, or derivatives thereof, preferably N-acetylcysteine.

The vanadium compounds or derivatives or analogues thereof, and optionally one or more antioxidants may be formulated into pharmaceutical compositions, either alone or together with other substances. A vanadium compound and antioxidant may be prepared and administered as a complex. For example, a vanadium compound may be complexed with glutathione or N-acetylcysteine.

The pharmaceutical compositions for arthropathy applications are delivered systemically. "Systemically" refers to a route of adminstration other than an intra-articular route, and includes oral, sublingual, intravenous, intraperitoneal, intraocular, intranasal, intradermal, topical, intravesical, intrathecal, intracranial, intramuscular or subcutaneous adminstration. A mode of administration may also be used which results in a slow continuous release of the active substances. This may be achieved by intravenous administration, subcutaneous administration, or using control release mechanisms such as implants or pumps. Control release methods generally use control release polymers and the release of the active ingredient is based on solubility properties, and the pore size of the polymers and active ingredients. The vanadium compounds such as vanadate or vanadyl compounds, may also be administered in pastes, such as thermopastes, in microspheres or palla beads. In a preferred embodiment of the invention, the vanadium compound and optionally antioxidant(s) are administered orally, intravenously, intranasally, subcutaneously, or intramuscularly.

The preparations of the invention can be intended for administration to humans and various other mammals, such as ovines, bovines, equines, swine, canines, and felines.

The compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the pharmaceutical compositions include, albeit not exclusively, solutions of the vanadium compounds such as vanadate or vanadyl compounds, derivatives or analogues thereof in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

A vanadium compound such as a vanadate or vanadyl compound may be administered to arthritic mammals for example at a dose of from 0.2 to 50 mg/kg, preferably from 0.5 to 20 mg/kg, most preferably from about 1 to 15 mg/kg. Appropriate doses may be determined in clinical trials. The antioxidant may be administered at a dose of from about 40

to 1,000 mg/kg, preferably from 75 to 250 mg/kg, most preferably about 100 mg/kg.

When the vanadium compound or analogue or derivative thereof is used in combination with one or more antioxidants, the doses of the vanadium compound or analogue or derivative thereof and the antioxidant(s) are selected so that the vanadium compound and antioxidant(s) alone would not show a full effect. Generally, the effective doses of the vanadium compound and the antioxidant(s) are the minimum doses adequate for enhanced anti-arthritic effects. The vanadium compound and antioxidant(s) may be administered concurrently, separately, or sequentially.

It is contemplated that the vanadium compound (e.g. a vanadate or vanadyl compound) or derivative, complex or analogue thereof may be administered in combination with other treatments for arthropathy or arthritis. Thus the methods of the invention for treating arthropathy may be used in combination with other treatment modalities known in the art. For the treatment of arthritis for example, the compounds may be administered in combination with, prior to, or subsequent to, other compounds known for use with arthritis, including methotrexate, cyclosporin, gold, penicilamine, plaquanil, non-steroidal anti-inflammatory agents, corticosteroids, anti-TNF, cyclophosphamide etc.

While not intending to be bound by any particular theory, it appears that the vanadium compound reduces joint pathology by lowering intracellular hydrogen peroxide levels and suppressing *fos/jun* and collagenase expression, which may be elevated in the disease state in response to such factors as interleukin-1. Collagenase degrades collagen, and increased collagenase expression is associated with cartilage erosion and synovitis in developing arthritis. Collagenase is one of the matrix metalloproteinases which have been associated with the breakdown of cartilage. The administration of a vanadium compound in the methods of the invention has been shown by the inventor to suppress the levels of other matrix metalloproteinases, including stromelysin, which degrades procollagens, and may also suppress other proteinases such as gelatinase, matrilysin and metalloelastase.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

Example 1

Treatment of Collagen Induced Arthritis by bis(maltolato)oxovanadium and N-acetylcysteine

The effects of treating rats having collagen induced arthritis with bis(maltolato)oxovanadium BMOV and N-acetylcysteine were investigated as follows. The chemical structure of BMOV is shown in Figure 1.Syngeneic 8 week old female Louvain (LOU) rats were fed with standard lab chow and housed in the vivarium at the University of California, Los Angeles. Arthritis was induced in the rats by intradermal immunization under ether anesthesia on Day 0 with 0.5 mg native chick collagen II (CII) (Genzyme, Boston, MA) solubilized in 0.1 M acetic acid and emulsified in incomplete Freund's adjuvant (IFA)

(Difco, Detroit, MI) (Trentham, D.E. et al., J. Exp. Med., 146: 857-868, 1977). Onset of clinical arthritis, characterized by erythema and edema in the hind joints, typically developed in 90-100 % of control rats 10-12 days post CII immunization.

Rats with definite arthritis on Day 10 post immunization were randomized into two groups. Control rats (n=8) received only NAC at a dose of 100 mg/kg/day s.c. NAC was prepared as a 3% aqueous solution. The experimental group (n=9) received NAC at 100 mg/kg/day s.c., as well as BMOV subcutaneously at 10 mg/kg/day. BMOV was solubilized in a 5% dextrose solution at 50°C. On Day 11 post arthritis onset, the dose of BMOV was increased to 15 mg/kg/day, because of local injection site sclerosis and concerns about bioavailability, and maintained throughout the rest of the study period.

Clinical arthritis severity of each limb was scored daily based on an objective integer scale of 0-4 (Trentham, D.E. et al., J. Exp. Med., 146: 857-868, 1977). A score of 0 indicated an unaffected limb, while a score of 4 represented fulminant erythema and edema involving distal digits. The arthritic index of a rat is defined as the sum of its four limb scores. Since CIA typically involves only the hind limbs, an arthritic index of 6 to 8 is considered severe arthritis.

Radiographs of the hind limbs were obtained at the end of the experiment on Day 18 post arthritis onset. An investigator blinded to the treatment protocol assigned a score to each limb, based on the degree of soft tissue swelling, joint space narrowing, periosteal new bone formation, and the presence of erosions and/or ankylosis (0=normal; 3=maximal joint destruction). Each rat had a maximal possible radiographic index of 6.

Humoral immunity was evaluated as follows. Rat serum was collected on Day 18 post arthritis onset to measure anti-CII IgG by an enzyme linked immunosorbent assay (ELISA) (Brahn, E. and Trentham, D.E., Cell Immunol., 86: 421-428, 1984; Brahn, E. and Trentham, D.E., Cell. Immunol., 118:491-503, 1989). Antibody titers, determined in quadruplicates, were normalized against a previously standardized curve and were expressed as the absorbance at 490 nm at a serum dilution of 1:2500.

Collagenase, stromelysin, and IL-1 expression were quantitated in each group of rats. Selected rats in each group were sacrificed on Day 18 post arthritis onset to measure collagenase, stromelysin, and IL-1 expression via the Northern Blot. Synovial biopsies were pooled and homogenized in the presence of RNASTAT-60 (Tel Test). Total RNA was isolated following the manufacturer's instructions, washed in 70% ethanol, and dissolved in 30 µl RNA loading buffer (Sigma) containing ethidium bromide. The RNA was electrophoresed on a 1% agarose formaldehyde gel and transferred to 0.45 µm nylon filter membrane (Magna NT, MSL). The blot was prehybridised in 50% formamide, 5x SSPE, 5x dendhardts, 1% SDS, 200 µg/ml ssDNA and 50 µg/ml tRNA. The rat collagenase cDNA (bp. 1-550 of locus RATCOL Genbank accession M60616), rat stromelysin (ATCC, Rockville, MD), or rat IL-1a (Genbank accession D00403) were labeled by random primed incorporation of ^{32}P-dATP (Random Primed

Labeling Kit, Boerhinger Manheim). After overnight hybridization at 42°C overnight, the blot was washed in 1x SSPE at 37°C and exposed to Kodak X-Ornat AP film for 24 hours at -70°C with an intensifying screen. The blot was stripped with 50% formamide in 2xSSPE, checked for residual counts and reprobed. The resulting autoradiographs were digitized and analyzed with NIH image software and normalized for RNA loading.

The synovium of rats from each group was examined by electron microscopy. Rats in each group were selected on Day 5 and Day 18 post arthritis onset to study joint morphology. Scanning and Transmission electron microscopy was performed on selected glutaraldehyde fixed joints. One ankle joint of each arthritic control and BMOV-treated rat was removed, critical point dried, and gold sputter-coated for scanning electron microscopy on Day 18 post arthritis onset to examine the trochlear surfaces. Conventional transmission electron microscopy was also performed on the articular cartilage of the trochlear surfaces of naive, arthritic control, and BMOV-treated animals using a Jeol 1200EX.

Other rats were anesthetized with Halothane (Halocarbon Laboratories, River Edge, NJ) and perfused 4 minutes with 5 ml of the Mercox polymer (2.5 g Mercox/0.1 g benzoyl peroxide; Mercox was a generous gift of Dr. Larry Arsenault, MacMaster University, Ontario, Canada). After allowing the Mercox to polymerize at room temperature for one hour, the hind limbs were harvested and placed in an oven for an additional hour at 60°C to complete polymerization. They were then placed in a 20% NaOH solution to digest tissues. The corrosion cast after the NaOH digestion, consisting of a Mercox polymer cast of blood vessels, was examined by scanning electron microscopy for capillary morphology. Student's t-test was used to analyze experimental data, and $p < 0.05$ was considered significant.

The daily mean arthritis score is shown in Figure 2. Rats given BMOV and NAC demonstrated significant regression of established arthritis compared to controls within two days post arthritis onset ($p<0.05$) (Figure 2). Control rats, receiving NAC alone, developed severe arthritis, a result suggesting that the reducing agent per se did not modify arthritis development significantly. The difference between the mean daily arthritis scores of the control and the experimental groups remained significant throughout the rest of the study period ($p<0.005$ on Day 18 post arthritis onset). The mean radiologic scores of the experimental group was significantly lower than the control group ($p<0.005$) (Table I and Figure 2). All experimental rats tolerated the combination of BMOV and NAC without weight loss. Diarrhea was not observed when BMOV was given at a dose of 10 mg/kg/day. However, when the dose was increased to 15 mg/kg/day on Day 11 post arthritis onset, a few experimental rats manifested minor diarrhea.

The mean anti-CII IgG titer of the control group was significantly higher than that of the experimental group ($p<0.04$). The biological significance of this difference, however, remained unclear since the magnitude of the difference was minimal and previous experiments have shown that arthritic rats often produce higher titers of anti-CII IgG than

nonarthritic rats.

X-rays of control and experimental rat limbs are shown in Figure 3A and 3B. A typical arthritic control limb is shown in Figure 3A and illustrates the soft tissue swelling and bone erosion. These features are absent in the vanadate treated experimental limb, shown in the right panel of Figure 3B.

The articular cartilage of control rats is shown in scanning electron micrographs in Figures 4A, 4B, 4C, 4D, 4E and 4F. In arthritic control rats (4A, 4C, and 4E), the articular cartilage of the trochlear surface is characteristically scabrous with an excessive number of erosion sites, pits (arrows) and adhering cells (C). In contrast, the BMOV-treated rats (4B, 4D, and 4F) exhibited a normal trochlear surface characterized by scant adhering elements (arrows) and a smooth articular surface with orderly arranged collagen fibrils (arrowheads). Articular surface was mechanically damaged during dissection (*). The magnifications are 25x for Figures 4A and 4B (Bar=1 mm), 260x for Figures 4C and 4D (Bar=100 μm), and 1700x for Figures 4E and 4F (Bar=10 μm).

Transmission electron micrographs showing trochlear articular cartilage from naive, arthritic control, and BMOV-treated rats are shown in Figures 5A, 5B and 5C respectively. The typical ultrastructure of the naive animal (Figure 5A) is contrasted with that of the arthritic control (Figure 5B) having its articular surface overgrown with cells (C) and pitted surface (*). On the other hand, the articular cartilage of BMOV-treated animals (Figure 28C) appeared indistinguishable from the naive animal. (For Figures 5A, 5B and 5C Bar=10 μm).

The scanning and transmission electron micrographs demonstrated dramatic cartilage destruction in the control joints (Figures 4A, 4C, 4E and 5B) with exposed or absent chondrocytes in the denuded cartilage. Joints from BMOV-treated rats demonstrated little cartilage damage and intact cartilage (Figures 4B, 4D and 4F).

Northern blots of collagenase, stromelysin and IL-1 expression are shown in Figure 6. Synovial expression of collagenase, stromelysin, and to a lesser degree, IL-1, were reduced in the BMOV group compared to the control group (Figure 6). Collagenase, stromelysin, and IL-1a mRNA were readily detected in the vehicle control group. When normalized for RNA loading, expression of all three genes was decreased in the animals in the BMOV-treated group compared to the control group. The percent inhibition of collagenase, stromelysin, and IL-1a gene expression were 78%, 58%, and 85%, respectively.

The results show that the combination of BMOV and NAC significantly regressed established CIA, compared to the control using NAC alone, by both clinical and radiologic criteria. The results indicate that the combination of vanadate and NAC regressed established CIA via decreasing collagenase expression. Collagenase mRNA expression in control arthritic rats were significantly higher than that in combination treated nonarthritic rats. Furthermore, the scanning electron micrographs showed much erosion in the synovium of

control joints, with chondrocytes exposed to the synovial surface. In contrast, the surface of combination treated synovium had a smooth appearance without chondrocytes exposed. The single agent NAC had no appreciable effect on the clinical severity of CIA. The combination of vanadate and NAC demonstrated efficacy at regressing established CIA due to at least two molecular mechanisms: decreased collagenase gene expression and decreased hydrogen peroxide concentration.

Example 2

Cytotoxicity Assays

The relative cytotoxicity of various compounds on tumor cell lines was investigated as follows. Cytotoxicity was measured by the MTT microculture tetrazolium colorimetric assay for all cell lines. The relative cytotoxicity of Na_3VO_4 and $VOSO_4$ was investigated using the following tumor cell lines: P388(WT) murine leukemia, P388 (ADR) murine leukemia, Lewis murine lung, MCF7 (WT) human breast, H460 human lung, K562 human erythroleukemia, A431 human epidermal, LS180 human colon and SK-OV-3 human ovarian. The relative cytotoxicity of doxorubicin, Na_3VO_4 and $VOSO_4$ was investigated using the following tumor cell lines: P388(WT) murine leukemia, P388 (ADR) murine leukemia, MCF7(WT) human breast and MCF7 (ADR) human breast. The relative cytotoxicity of BMOV, BEOV and naglivan was investigated using the following tumor cell lines: P388(WT) murine leukemia, P388 (ADR) murine leukemia, Lewis murine lung, H460 human lung, K562 human erythroleukemia, and SK-OV-3 human ovarian.

The cells were plated (number of cells/well was different from cell line to cell depending on the dividing rate) in a 96-well microculture plate 24 hours prior to the delivery of the vanadium compounds. Serial concentrations of solutions of the test compounds (0.05 - 100 μM) were delivered to the corresponding wells. A blank column (without cells) and a control column (with cells without vanadium) were left. The cells were incubated for 72 hours continuously. An MTT solution was added to each well after this incubation period and the cells were incubated for 4 more hours for the completion of the dyeing process. The medium was removed and DMSO was added to each well. The absorbance of each well was read with a Titertek Multiskan (310C) spectrophotometer at 570 nm. The percentage of the absorbance of each vanadium concentration relative to the control calculated and the value of the 50% growth inhibition concentration (IC^{50}) was obtained from the % control versus concentration plot. Each assay was repeated three times and the reported IC^{50} values were the mean of these three runs.

The relative cytotoxicity of Na_3VO_4 and $VOSO_4$ on tumor cell lines is shown in Table 1. The relative cytotoxicity of doxorubicin, Na_3VO_4 and $VOSO_4$ on drug sensitive and MDR tumor cell lines is shown in Table 2. The relative cytotoxicity of BMOV, BEOV and naglivan on tumor cell lines is shown in Table 3.

Example 3

The Effect f thermopaste of vanadyl sulfate and MePEG/PCL on tumor growth

The effect of vanadyl compounds in pastes, or in microspheres, on tumor growth was investigated to assess whether the thermopaste of vanadyl sulfate is effective in reducing tumor growth and to examine the optimum concentration of vanadyl sulfate loaded in the polymer. Forty mice were subcutaneously injected with MDAY-2D tumor cells ($3.6x10^5$/100µl) on day 1. On day 5, the mice were divided into six groups and received implanted polymer/drug thermopaste. The mice received the following treatments: Group 1: empty control, Group 2: polymer alone (no drug loaded), Group 3, 4, and 5: 5%, 10%, 20%, 30% vanadyl sulfate loaded in polymers respectively.

The results are shown in Table 4. The mice in group 5 (20% vanadyl sulfate) and group 6 (30% vanadyl sulfate) died in the third day post-operation. The mice were sacrificed on the day 18 post-operation. The tumors and remained polymers were dissected and weighted. The results showed that the thermopaste of vanadyl sulfate/polymer is effective in significantly reducing the tumor growth. The thermopaste of vanadyl-sulfate/MePEG/PCL was effective in reducing tumor growth. The concentrations of 20% and 30% vanadyl sulfate loaded in the polymer were likely too high because all the mice died in the two groups.

Example 4

The Effect of BMOV Loaded Microspheres on Tumor Growth in Mice

The effect of slow releasing microspheres loaded with 20% BMOV on tumor growth in mice was investigated as follows. Twenty of Twenty-four mice were injected subcutaneously with 100 ml of MDAY-D2 cells with density of 10×10^6/ml. On day 6 the mice were divided into 6 groups as follows: group 1, empty control; group 3, tumor control; group 3, injected subcutaneously with 0.25 mg/100µl BMOV, twice a day; group 4, injected IP with 20 mg palla beads containing 5 mg of BMOV; group 5, injected IP with 10 mg of BMOV beads on day 6 and day 9 respectively and; group 6, injected intramuscularly with 10 mg of BMOV beads on day 6 and day 9. On day 16, the mice were sacrificed and tumors were dissected.

The body weights of mice in control and treated groups, and the tumor weights from control and treated mice, are shown in Tables 5 and 6 respectively. The results show that BMOV loaded microspheres were effective in reducing tumor growth when administered IP.

Example 5

The Effect of Thermopastes (PCL/PLA) Loaded with BMOV on Tumor Growth

The effectiveness of thermopaste containing BMOV in reducing tumor growth wasinvestigated as follows. Forty mice were subcutaneously injected with tumor cells ($10x10^6$/ml) on day 1. On day 6, the mice were divided into six groups and implanted with thermopaste. Group 1 mice received control thermopaste (PCL/PLA) alone, group 2 mice received PCL alone, group 3 and 4 mice received 30% and 35% BMOV respectively loaded in

PCL.

The resulting tumor weights in the different groups of mice are shown in Table 7. The results showed that that the thermopastes loaded with BMOV are effective in reducing tumor growth.

Example 6

The Effect of Combination use of BEOV and N-acetylcysteine on Tumor Growth

The effectiveness of BEOV in reducing tumor growth was investigated and the effective concentration required for inhibiting tumor growth was determined as follows. Forty mice were subcutaneously injected with 100 µl of MDAY-2D tumor cell suspension ($8x10^6$/100 µl)on day 1. On day 6, the mice were randomly divided into six groups and injected following drugs twice a day. The six groups were as follows: Group 1: 100 µl of PBS; Group 2: 100 µl of 2% NAC(ip) and 0.1 mg BEOV in 100 µl BS; Group 3: 100 µl of 2% NAC(ip) and 0.25 mg BEOV in 100 µl PBS; and Group 4: 100 µl of 2% NAC(ip) and 0.5 mg BEOV in 100 µl PBS. On injection day 9, the mice were sacrificed and the tumors dissected. The resulting tumor weights of mice in the different groups are shown in Table 8 and the body weights are shown in Table 9. The effect of BEOV on tumor growth is shown in Figure 7. The data shows that BEOV used in combination with NAC inhibited the tumor growth at concentrations of 0.25 mg to 0.5 mg BEOV per day.

Example 7

The Effect of Combination use of Ammonium Bisvanadate(ABOV) N-acetylcystine on tumor growth

The following experiments were carried out to assess whether ammonium bisvanadate is effective in reducing tumor growth while being less toxic, and to examine the effective concentration of the reagent for inhibiting tumor growth. Forty mice were subcutaneously injected with MDAY-2D tumor cells ($8x10^5$/100 µl) on day 1. On day 6, the mice were randomly divided into six groups and injected with the following drugs twice a day: Group 1: 100 µl of PBS; Group 2: 100 µl of 2% NAC(ip) and 0.1 mg ammonium bisvanadate in 100 µl PBS; Group 3: 100 µl of 2% NAC(ip) and 0.25mg ammonium bisvanadate in 100 µl PBS; Group 4: 100 µl of 2% NAC(ip) and 0.5 mg ammonium bisvanadate in 100 µl PBS; Group 5: 100 µl of 2% NAC (ip) and 0.75 mg ammonium bisvanadate in 100 µl PBS and; Group 6: 100 µl of 2% NAC (ip) and 1.0 mg ammonium bisvanadate. On injection day 11 the mice were sacrificed and the tumors dissected. The tumor weights in the different groups are shown in Table 10. The effect of orthovanadate concentration is shown in Figure 8. The results show that the ammonium bisvanadate and NAC inhibited the tumor growth in mice. The effective concentrations of the vanadate complexes are from 0.25mg/100 µl and 0.5 mg/100 µl.

Example 8

The Effect of Different Concentrations of BMOV on Tumor Growth

The previous examples showed that BMOV and N-acetylcystine can effectively

reduce tumor growth. In this experiment we determined the optimum concentration for inhibition of tumor growth as follows. Forty mice were subcutaneously injected with tumor cells ($10x10^6$/ml)on day 1. On day 5, the mice were divided into five groups as follows: Group 1: control, PBS injected; Group 2: 0.1% BMOV injected; Group 3: 0.25% BMOV injected; Group 4: 0.5% BMOV injected and; Group 5: 0.75% BMOV injected. All the mice were injected (I.P.) with 2 mg NAC before injection of BMOV. The tumor weights in the different groups are shown in Table 11. The effect of BMOV concentration is shown in Figure 9. The results show that the BMOV and NAC inhibited the tumor growth in mice. The effective concentrations of BMOV are from 0.1% to 0.75%.

Example 9

The Effect of Orthovanadate and Vanadyl Sulphate on Synoviocyte Proliferation

The effect of vanadium compounds on synoviocyte proliferation was investigated as follows. Synoviocytes were plated on six well plates and treated with orthovanadate or vanadyl sulphate and incubated for 24 hours. The cells were removed and the number of viable cells determined by the dye exclusion method. The results are shown in Table 12. Both orthovanadate and vanadyl sulphate treatment inhibited synoviocyte proliferation and were cytotoxic to the cells.

Example 10

The efficacy of vanadium compounds conjugated with hydrophobic structures [e.g. orthovanadate bis(ethylmaltolato) oxovanadium (BEOV), ammonium bis vanadate (ABOV) and bis(methylmaltolato) oxovanadium (BMOV)] was investigated. BEOV was found to be the most effective agent in reducing cell proliferation i.e. tumor growth relative to other vanadium complexes in an MDAY tumor model (Table 13). The toxicity (body weight loss) observed with BEOV and BMOVwas similar or less than that observed with other vanadium compounds and the animals tolerated significantly higher doses. To further assess the efficacy of BEOV, the compound was tested in split dosing studies. BEOV significantly decreased cell proliferation i.e. tumor growth rate at doses which demonstrated minimal organ toxicity. In summary, tumors were absent in approximately 75% of mice treated with BEOV, compared with 38% of the mice being tumor free when they were administered BMOV.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated by those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. I claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

TABLE 1

Table 1: Relative Cytotoxicity of Na_3VO_4 and $VOSO_4$ on Tumor Cell Lines.

Cell line	Type	Exposure Time (hours)	IC_{50} Value (μM)	
			Na_3VO_4	$VOSO_4$
P388 (WT)	Murine leukemia	72	5.5	16.1
P388 (ADR)	Murine leukemia	72	14.8	29.8
Lewis lung	Murine lung	72	42.3	27.3
MCF7 (WT)	Human breast	72	8.5	10.5
MCF7 (ADR)	Human breast	72	30.7	31.3
H460	Human lung	72	1.5	9.3
K562	Human erythroleukemia	72	100	>100
A431	Human epidermal	72	7.6	29.1
LS180	Human colon	72	16.4	31.9
SK-OV-3	Human overian	72	2.0	1.9

TABLE 2

Table 2. Relative Cytotoxicity of Different Compounds on Drug Sensitive and MDR Tumor Cell Lines.

Cell line	Type	Exposure Time (hours)	IC_{50} Value (μM)		
			doxorubicin	Na_3VO_4	$VOSO_4$
P388 (WT)	Murine leukemia	72	0.023	5.5	16.1
P388 (ADR)	Murine leukemia	72	3.771	14.8	29.8
MCF7 (WT)	Human breast	72	0.492	8.5	10.5
MCF7 (ADR)	Human breast	72	25.74	30.7	31.3

TABLE 3

Table 3. Relative Cytotoxicity of Vanadium Compounds on Tumour Cell Lines.

Cell Lines	Type	Exposure Time (hours)	Drug IC_{50} (μM)		
			BMOV	BEOV	naglivan
SK-OV-3	Human ovarian cancer	72	2.6	2.6	2.5
H460	Human lung cancer	72	6.4	4.4	6.1
K562	Human erthroleukemia	72	>1000	374	4.7
P388 (WT)	Murine leukemia	72	31.1	82.6	4.6
P388 (ADR)	Murine leukemia	72	61.2	39.8	5.5
Lewis lung	Murine lung cancer	72	142.2	97.4	7.0

TABLE 4

Tumour weights (gm) of different groups

	Empty control	Polymer (no drug)	5% VOS_4	10% VOS_4
1	0.94	0.88 (+0.16)	0.11 (+0.0)	0.41 (+0.0)
2	0.78	2.66 (+0.14)	0.10 (+0.0)	0.48 (+0.15)
3	1.25	0.98 (+0.14)	0.42 (+0.15)	0.76 (+0.15)
4	1.62	1.45 (+0.12)	0.13 (+0.10)	0.22 (+0.0)
5	0.93	0.56 (+0.13)	died	0.00 (+0.0)
6	1.10	4.92 (+0.02)		
7	1.67	0.98 (+0.16)		
8	1.46	1.39 (+0.15)		
9	1.37	1.15 (+0.16)		
10	1.44	0.57 (+0.15)		
Mean	1.261	1.355	0.190	0.374
Std. dev	0.3019	0.8138	0.153	0.2851
P value		0.003	0.001	0.005

TABLE 5

Body weights of mice in control and treated groups

	Control	Tumour Control	B M O V Solution 0.25mgx1	B M O V beads, IP 5mg/once	B M OV beads, IP 2mgx2	B M O V beads, IM 2mgx2
1	19	23.9	18	18.8	19.2	18.2
2	20.2	21	17.8	19.3	18.9	22.6
3	18.4	20	18.4	21.6	17.2	20.1
4	22.2	24.1	17.3		19.1	22.2
Average	19.95	22.25	17.87	19.9	18.6	20.78

TABLE 6

Tumour weights of control and treated groups

	Tumour control	B M O V solution, IP	B M O V beads, IP 5mg/once	B M O V beads, IP 2mgx2	B M O V beads, IM 2mgx2
1	3.8	0.4	0.2	0.12	2.0
2	1.2	0.12	0.9	0.4	3.3
3	1.3	0.07	0.26	0.3	1.1
4	1.5	0.04	died	1.2	1.6
Average	1.95	0.15	0.45	0.50	2.0

TABLE 7

Tumour weights in different groups

Group	PCL	30%BMOV PCL	35%BMOV PCL
1	1.15 (0.06)	0.02 (0.18)	0.36 (0.19)
2	1.12 (0.07)	0.17 (0.18)	0.50 (0.18)
3	1.04 (0.12)	0.13 (0.16)	0.15 (0.16)
4	2.05 (0.14)	1.40 (0.17)	0.69 (0.19)
5	1.82 (0.12)	0.37 (0.16)	0.16 (0.17)
6	2.25 (0.09)	0.20 (0.16)	0.0 (0.16) one died
Means:	1.57	0.38	0.31

TABLE 8

Tumour weights in groups

No.	1 Contr. PBS	2 0.1 mg BEOV	3 0.25 mg BEOV	4 0.5 mg BEOV
1	2.2	0.9	0.28	0.1
2	1.7	0.6	0.0	0.0
3	1.4	0.8	0.0	0.0
4	0.9	1.0	0.12	0.0
5	1.0	died	0.0	died
Average	1.44	0.82	0.08	0.03

TABLE 9

Body weight of mice in different group

No.	Contr. PBS	0.1 mg BEOV	0.25 mg BEOV	0.5 mg BEOV
1	16.9	17.8	15.9	13.7
2	18.8	15.5	15.7	13.9
3	18.2	15.9	16.9	16
4	16.6	16.8	15.4	17.4
5	19.1	died	16.2	died
Average	18.12	16.5	16.02	15.25

TABLE 10

Tumour weights in groups

Group	1	2	3	4	5	6
	Contr.	0.1mg ABOV	0.25mg ABOV	0.5mg ABOV	0.75mg ABOV	1.0mg ABOV
1	0.94	0.62	0.25	0.21	died	died
2	0.84	0.19	0.33	0.50	-	-
3	1.21	0.74	0.54	0.07	-	-
4	1.02	0.53	0.20	0.34	-	-
5	1.56	0.32	0.0	0.0	-	-
6	1.14	0.50	0.18	0.0	-	-

TABLE 11

	Tumour weights				
	Contr.	0.1%	0.25%	0.5%	0.75%
1	1.6	0.44	0.14	0.40	0.07
2	1.07	0.40	0.23	0.20	0.16
3	0.92	0.48	0.73	0.07	0.08
4	0.95	0.62	0.04	0.17	0.21
5	1.04	0.54	0.21	0.06	0.13
6	1.01	0.84	0	0	0
7	1.00	0.18	0	0	0
8	--	0.87	0	0	died
Means	1.08	0.55	0.18	0.22	0.09

TABLE 12

Treatment	Number of cells (10^{-5})
Control	2.6
Orthovanadate (25 μM)	0.1
Vanadyl Sulphate (25 μM)	0.2

TABLE 13

Efficacy and Toxicity of Vanadium Compounds

Compound	Efficacy		Toxicity
	% Inhibition of tumor growth	% non-detectable	LD_{50}
VSO_4	73	8	0.75 mg
VO_4	89	34	0.75 mg
ABOV	88	33	<0.75 mg
BMOV	92	38	>0.75 mg
BEOV	99	75	>0.75 mg

I CLAIM:

1. A method for treating a mammal having an arthropathy, comprising systemically administering to the mammal an amount of a vanadium compound effective to reduce or inhibit the arthropathy, with the proviso that the vanadium compound is not bis(methylmaltolato) oxovanadium (BMOV).

2. A method for treating a mammal having an arthropathy, comprising systemically administering to the mammal an amount of a vanadium compound selected from the goup consisting of orthovanadate, sodium vanadate, vandyl sulfate, bis(ethylmaltolato) oxovandium (BEOV), and ammonium bisvanadate (ABOV), effective to reduce or inhibit the arthropathy.

3. A method as claimed in claim 1 or 2 wherein the method further comprises administering an antioxidant to the mammal.

4. A method as claimed in claim 1 or 2 wherein the vanadium compound is bis(ethylmaltolato) oxovandium (BEOV).

5. A method as claimed in claim 1 or 2 wherein the vanadium compound is ammonium bisvanadate.

6. A method as claimed in any one of claims 1 to 5 wherein the arthropathy is arthritis.

7. A method as claimed in claim 3 wherein the antioxidant is N-acetylcysteine.

8. A method for treating arthritis in a mammal comprising administering a pharmaceutical composition comprising an effective amount of a vanadium compound and a pharmaceutically acceptable vehicle, with the proviso that the vanadium compound is not bis(methylmaltolato) oxovanadium (BMOV).

9. A method for treating arthritis in a mammal comprising administering a pharmaceutical composition comprising an effective amount of a vanadium compound selected from the goup consisting of orthovanadate, sodium vanadate, vandyl sulfate, bis(ethylmaltolato) oxovandium (BEOV), and ammonium bisvanadate (ABOV) and a pharmaceutically acceptable vehicle).

10. A method as claimed in claim 8 or 9, wherein the pharmaceutical composition further

comprises at least one antioxidant.

11. A method for reducing proliferation of synoviocytes in a mammal comprising administering a vanadium compound in an amount effective to reduce the collagenase expression, with the proviso that the vanadium compound is not bis(methylmaltolato) oxovanadium (BMOV).

12. A method as claimed in claim 11, further comprising administering at least one antioxidant.

13. A method as claimed in claim 11 wherein the vanadium compound is selected from the goup consisting of orthovanadate, sodium vanadate, vandyl sulfate, bis(ethylmaltolato) oxovandium (BEOV), and ammonium bisvanadate (ABOV).

14. A pharmaceutical composition for use as an anti-arthritic agent comprising at least one of bis(ethylmaltolato) oxovandium (BEOV) and ammonium bisvanadate (ABOV), at least one antioxidant, and a pharmaceutically acceptable vehicle.

15. Use of a vanadium compound in the preparation of a pharmaceutical composition for treating an arthropathy, with the proviso that the vanadium compound is not bis(methylmaltolato) oxovanadium (BMOV).

16. Use of a vanadium compound selected from the goup consisting of orthovanadate, sodium vanadate, vandyl sulfate, bis(ethylmaltolato) oxovandium (BEOV), and ammonium bisvanadate (ABOV) in the preparation of a pharmaceutical composition for treating an arthropathy.

17. A use as claimed in claim 15 or 16 wherein the method further comprises administering an antioxidant to the mammal.

18. A use as claimed in claim 16 wherein the vanadium compound is bis(ethylmaltolato) oxovandium (BEOV).

19. A use as claimed in claim 16 wherein the vanadium compound is ammonium bisvanadate.

20. A use as claimed in any one of claims 15 to 19 wherein the arthropathy is arthritis.

21. A use as claimed in claim 17 wherein the antioxidant is N-acetylcysteine.

22. A method for reducing cell proliferation and metalloprotease expression comprising administering a vanadium complex selected from the group consisting of (a) metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate; and (c) coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate or vanadyl sulfates.

23. A method for reducing or inhibiting the growth of drug resistant tumors comprising administering an amount of a vanadium complex selected from the group consisting of (a) metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate; and (c) coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate or vanadyl sulfates, effective to reduce or inhibit the growth of drug resistant tumors.

24. A method for reducing or inhibiting metastases comprising administering an amount of a vanadium complex selected from the group consisting of (a) metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate; and (c) coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate or vanadyl sulfate, effective to reduce or inhibit metastases.

25. A method as claimed in claim 22, 23, or 24 wherein the vanadium complex is bis(ethylmaltolato) oxovandium (BEOV).

26. A pharmaceutical composition for the treatment of proliferative disorders comprising an amount of a vanadium complex effective to reduce cell proliferation, and one or more of a pharmaceutically acceptable carrier, diluent, or excipient, wherein the vanadium complex is selected from the group consisting of (a) metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or

thiohydroxamate; and (c) coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate or vanadyl sulfate,

27. A method as claimed in claim 26 wherein the vanadium complex is bis(ethylmaltolato) oxovandium (BEOV).

28. Use of a vanadium complex selected from the group consisting of (a) metavanadate and orthovanadate complexes; (b) organo-vanadium compounds where vanadium is bound to an organic moiety that can form a five- or six- membered ring or, to an organic moiety such as hydroxamate, a-hydroxypyridinone, a-hydroxypyrone, a-amino acid, hydroxycarbonyl or thiohydroxamate; and (c) coordinate-covalent complexes of vanadyl and cysteine or a derivative thereof, vanadyl acetylacetonate or vanadyl sulfate, cysteine or a derivative thereof, vanadyl acetylacetonate and vanadyl sulfates, for reducing cell proliferation and metalloprotease expression, reducing or inhibiting drug resistant tumors, and/or reducing metastasis.

29. Use of a vanadium complex as claimed in claim 28, wherein the vanadium complex is bis(ethylmaltolato) oxovandium (BEOV).

FIGURE 1

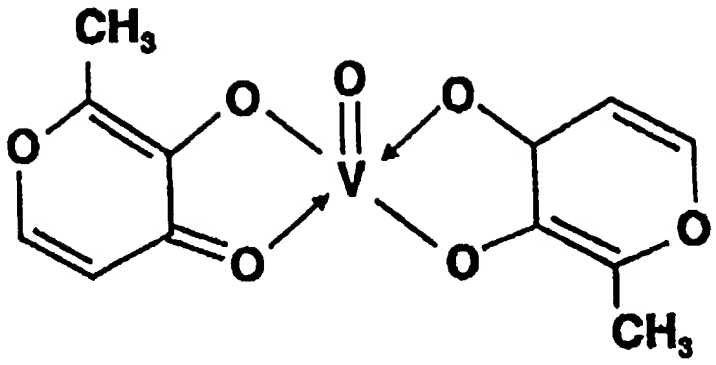

FIGURE 2

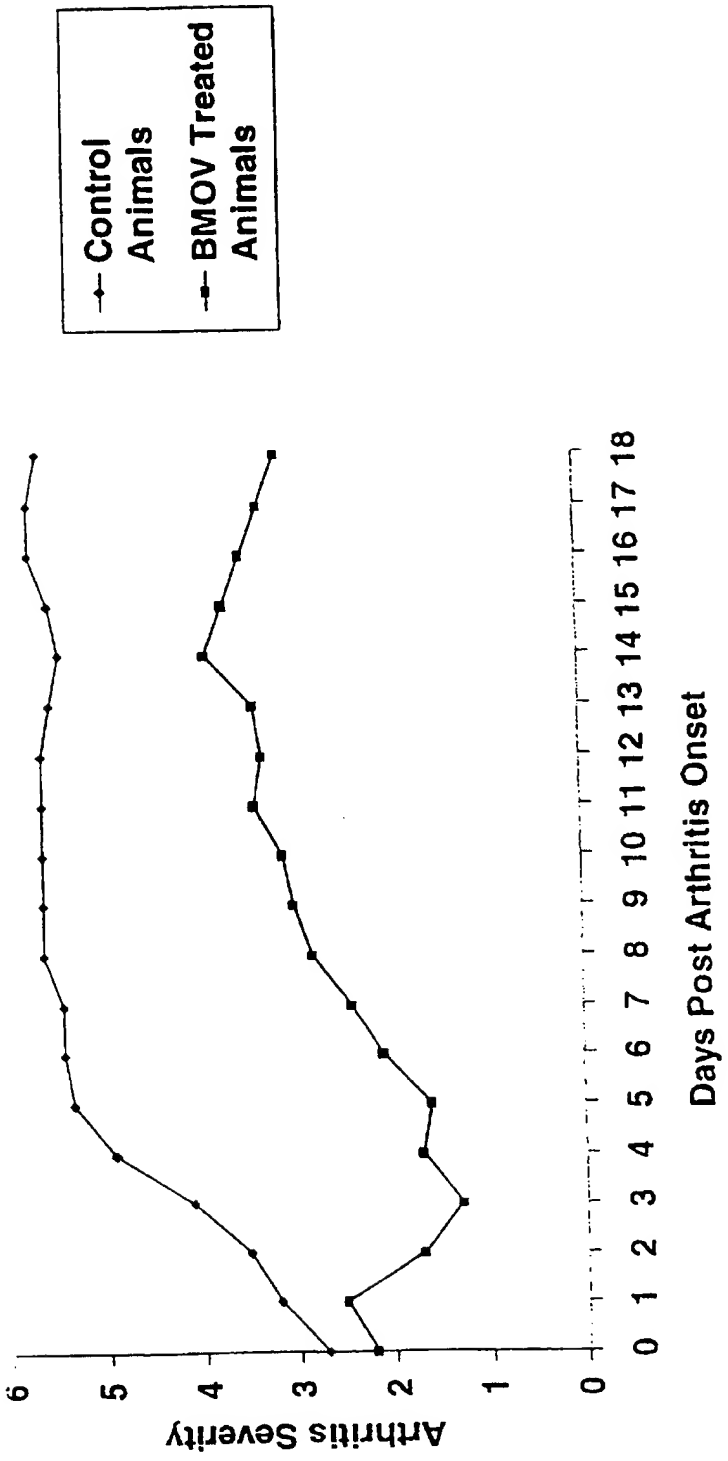
Control Animals
BMOV Treated Animals
Arthritis Severity
0
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Days Post Arthritis Onset

FIGURE 3A

FIGURE 3B

FIGURE 4A

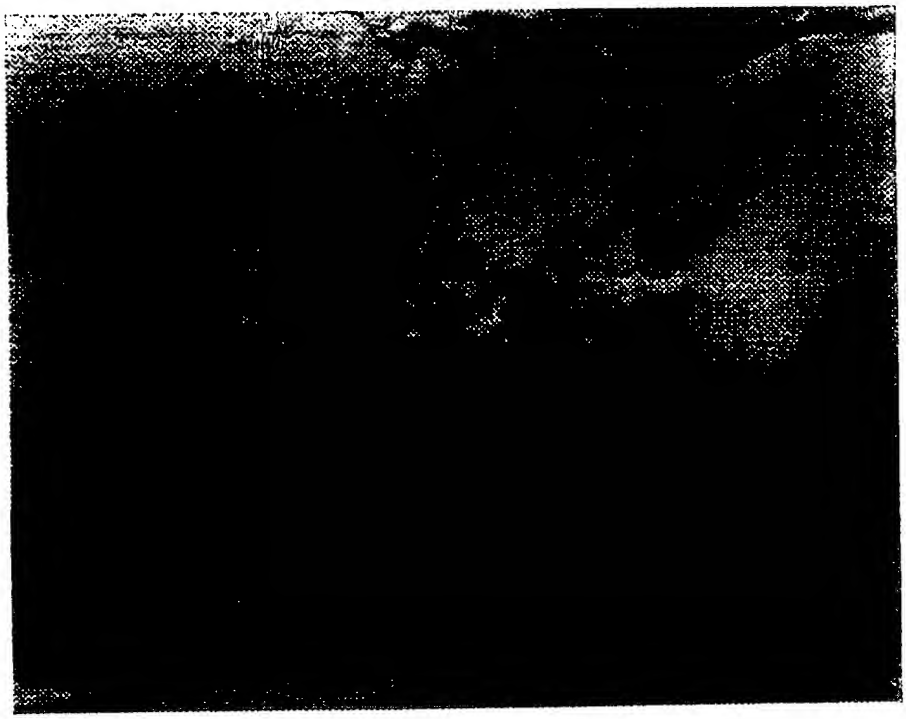

FIGURE 4B

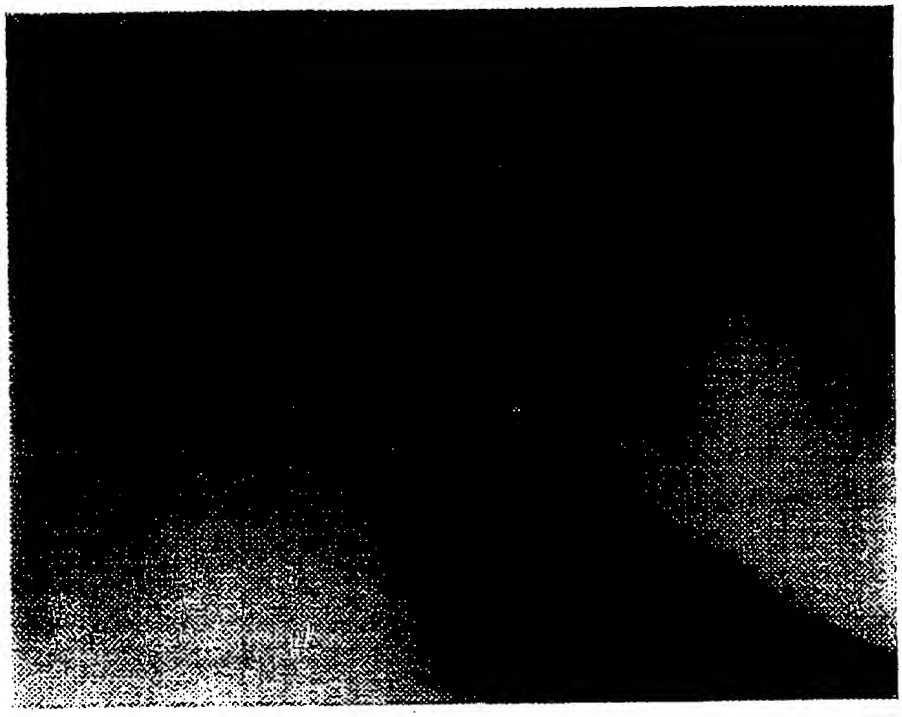

FIGURE 4C

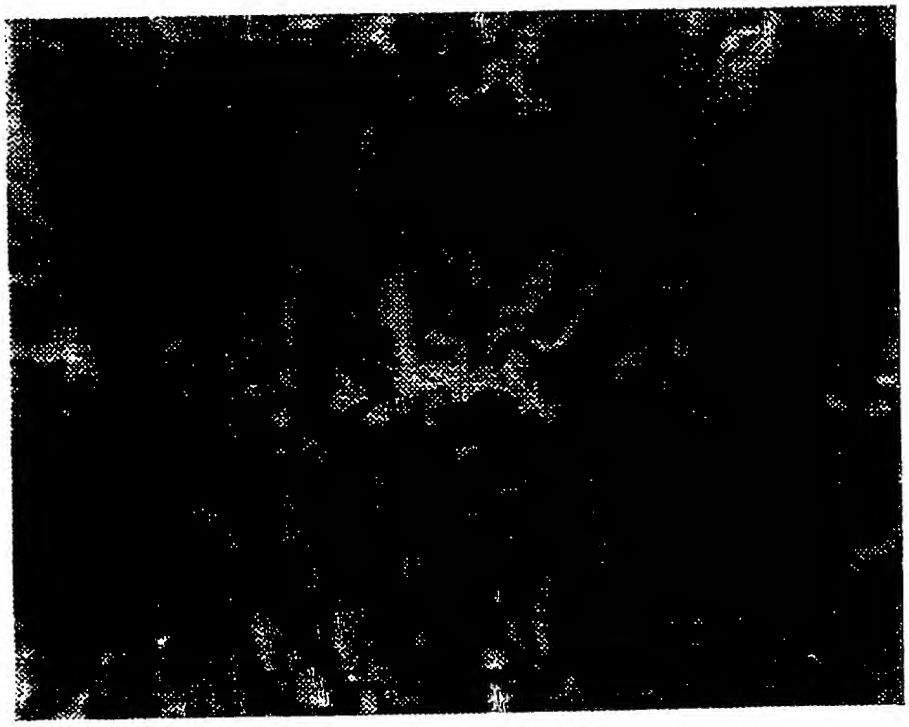

FIGURE 4D

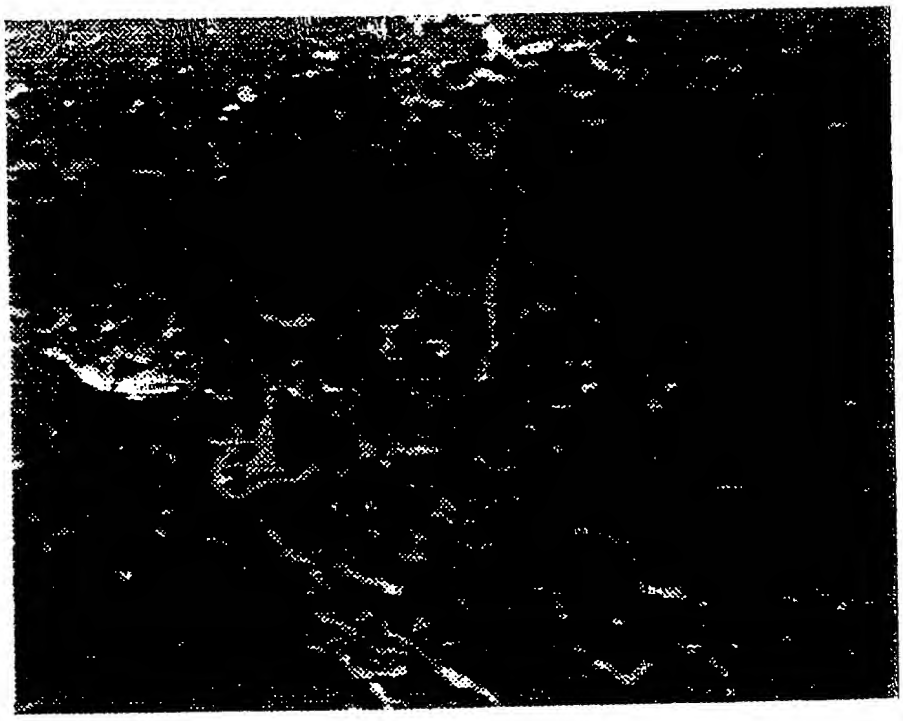

FIGURE 4E

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00405

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/28 A61K31/555 A61K33/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANUPAM BISHAYEE ET AL.: "Inhibition of altered liver cell foci and persistent nodule growth by vanadium during diethylnitrosamine-induced hepatocarcinogenesis in rats" ANTICANCER RESEARCH, vol. 15, no. 2, March 1995 - April 1995, GREECE, pages 455-461, XP002048167 see abstract see page 455, right-hand column see page 460, left-hand column, line 15 - page 461, left-hand column, line 10 --- -/--	22-29

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 1997	16. 12. 97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00405

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	TAMMY P.CHENG ET AL.: "Vanadate, an inhibitor of c-Fos, c-Jun, and collagenase expression, suppresses collagen arthritis" ARTHRITIS AND RHEUMATISM, vol. 38, no. 9 (SUPPLEMENT), September 1995, USA, page S372 XP002048168 see abstract	1-22, 25-29
X	C.DJORDJEVIC: "Antitumor activity and toxicity of peroxo heteroligand vanadates(V) in relation to biochemistry of vanadium" J.INORG.BIOCHEM., vol. 25, no. 1, September 1985, USA, page 51-55 XP002048169 see the whole document	22-29
X	S.SARDAR ET AL.: "Vanadium: a possible role in the protection of host cells bearing transplantable murine lymphoma" TUMOR RES., vol. 28, 1993, pages 51-61, XP002048170 see page 51, line 1 - page 52, line 26 see page 57, line 1 - page 59, line 3	22-29
P,X	J.K.JACKSON ET AL.: "A polymer-based drug delivery system for the antineoplastic agent bis(maltolato)oxovanadium in mice" BRITISH JOURNAL OF CANCER, vol. 75, no. 7, April 1997, pages 1014-1020, XP002048171 see abstract see page 1014, left-hand column, line 1 - page 1014, right-hand column, line 21 see page 1019, left-hand column, paragraph 2 - page 1020, left-hand column, line 36	22-29
X	CA 2 113 683 A (MOUNT SINAI HOSPITAL CO.) 19 July 1995 see page 1, line 6 - line 16 see page 4, line 7 - page 6, line 16 see page 7, line 35 - page 13, line 19 see page 15, line 17 - page 25, line 8 see claims 1-12	1-29
X	DD 293 495 A (AKADEMIE DER WISSENSCHAFTEN) 5 September 1991 see claims 1-3	26

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00405

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06811 A (THE UNIVERSITY OF BRITISH COLUMBIA) 15 April 1993 see page 3, line 11 - line 20 see page 3, line 29 - page 4, line 11 see page 4, line 18 - page 9, paragraph 4 see claims 1-22 ---	26
X	WO 95 20390 A (BRISTOL-MYERS SQUIBB CO.) 3 August 1995 see page 8, line 1 - line 18 see page 8, line 25 - page 10, line 20 see page 17, line 16 - page 36, line 36 see examples 1-15 see claims 1-116 ---	22-29
X	EP 0 305 264 A (PANMEDICA S.A.) 1 March 1989 see page 2, line 31 - page 3, line 4 see page 3, line 26 - page 5, line 49 -----	26

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00405

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CA 2113683 A	19-07-95	NONE	
DD 293495 A		NONE	
WO 9306811 A	15-04-93	US 5300496 A	05-04-94
		AU 2649792 A	03-05-93
		CA 2120338 A	15-04-93
		EP 0606318 A	20-07-94
		JP 6511244 T	15-12-94
		NZ 244569 A	27-04-95
		US 5527790 A	18-06-96
		US 5620967 A	15-04-97
		ZA 9207522 A	16-06-93
WO 9520390 A	03-08-95	US 5565491 A	15-10-96
		CA 2179715 A	03-08-95
		EP 0735880 A	09-10-96
		US 5583242 A	10-12-96
EP 0305264 A	01-03-89	FR 2619382 A	17-02-89
		CA 1315476 A	30-03-93
		DE 3878790 A	08-04-93
		ES 2054843 T	16-08-94
		JP 1066159 A	13-03-89
		US 5023358 A	11-06-91
		US 5175001 A	29-12-92